

Asbestos Fibers, Plasma and Inflammation

by John A. Hamilton*†

Fibrin clots have been detected at sites of inflammation, and kinins have been implicated as mediators of the vascular phenomena of acute inflammation, systemic shock, and disseminated intravascular coagulation. It is now reported that both negatively and positively charged asbestos fibers shorten the partial thromboplastin time of human plasma, indicating coagulation of the plasma. A sample containing short ($<5\ \mu\text{m}$ in length) chrysotile fibers is ineffective. Only the negatively charged amphiboles (crocidolite and amosite) are able to activate factor XII (Hageman factor). This particular effect of the amphiboles is enhanced by high molecular weight kininogen and leads to kinin formation.

Introduction

The critical features of asbestos fibers and the host responses to these structures, which are both responsible for the development of the inflammation associated with asbestosis, remain to be elucidated. Many negatively charged surfaces can cause plasma to clot, with activation of factor XII being an early step in the activation of the so-called intrinsic coagulation cascade (1). Fibrin clots have been detected at sites of inflammation (2), while several putative mediators of inflammation, for example, kinins and the products of the fibrinolytic pathway, can also result from the activation of factor XII (1). Since asbestos fibers have charged surfaces, the interaction of these fibers with human plasma was examined.

Materials and Methods

Samples

The asbestos and quartz samples were kindly provided by Dr. A. N. Rohl, Environmental Sciences Laboratory, Mt. Sinai School of Medicine.

Partial Thromboplastin Time (PTT)

PTT determinations were performed by standard techniques as described by Ratnoff and Davie (3). Fibers were suspended at 10 mg/mL in 0.1 M Tris-HCl,

0.1 M NaCl, pH 8.0, by sonication for 2 min. Test samples of 0.1 mL of fiber suspension or 0.1 mL of buffer control were incubated with 0.1 mL of 0.1% crude soybean phospholipid (Centrox-P, Chemistry Div., Central Soy, Chicago, IL). Kaolin (American Standard, acid-washed, Fisher Scientific Co., Toronto, Ontario) and quartz, at 10 mg/mL in Centrox-P, were used as control materials, as they are known to activate factor XII and thus the intrinsic clotting pathway (3). After this preincubation for 2 min at 37°C, 0.1 mL plasma substrate (ACD, acid-citrate-dextrose, normal human plasma from the Canadian Red Cross) was added by plastic pipet and the incubation continued at 37°C for an additional 8 min. Finally, 0.1 mL of 0.025 M calcium chloride was added as cofactor for the enzyme cascade system and formation of a solid clot was measured after the time of addition of the calcium chloride. All tests were carried out in duplicate. The clotting times are reproducible within 5-10%.

Factor XII Activation

Activation of factor XII was measured indirectly by the conversion of prekallikrein to kallikrein as described previously (4). Stock solutions of fiber samples (10 mg/mL) were prepared as described above. Activation of factor XII was measured indirectly as outlined previously (4). Activated factor XII converts prekallikrein to the protease kallikrein. The kallikrein was measured spectrophotometrically as arginine esterase activity using the synthetic ester benzoyl-L-arginine ethyl ester (BAEE) as substrate. Factor XII, HMW

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kininogen, and prekallikrein/kallikrein were purified as previously described (4, 5). A 0.1 mL portion of factor XII (1 mg) was incubated at 37°C with 0.1 mL fiber suspension or kaolin (10 mg/mL) for 2 min. Each reaction mixture was further incubated with 0.1 mL HMW kininogen (0.375 mg) or 0.1 mL buffer for 10 min. Each precipitate was washed and spun at 4°C (3050g for 10 min) and incubated with 0.1 mL prekallikrein (8.5 mg) for an additional 10 min. A 0.5 mL portion of each supernatant was added to 0.5 mL of 3 mM BAEe (Cyclo Chemical Corp., New York, NY) and made up to 3 mL with 0.1 M Tris-HCl, pH 8.0, containing 0.1 M NaCl. The absorbance due to released benzoyl arginine (BA) was read at 2-min intervals at 253 nm for 10 min (4). A mixture of 2.5 mL of Tris buffer and 0.5 mL (BAEe served as the blank. The increase in absorbance is expressed as nanomoles BAEe hydrolyzed per minute per milliliter of sample, with a standard curve of known amounts of BA.

Bioassay for Kinin-Forming Activity

Kinin generation after contact activation of factor XII was assayed by the ability to cause smooth muscle contraction of the rat uterus (5). A 0.1 mL portion of a IARC crocidolite suspension (10 mg/mL) was incubated with 0.1 mL normal human plasma (NHP), factor XII-deficient plasma (Hird), and HMW kininogen-deficient plasma (Williams) for varying lengths of time. The mixtures were then boiled for 5 min, quickly cooled, centrifuged at 4°C for 10 min at 3050g, and assayed for kinin activity by the ability to cause smooth muscle contraction of the estrous rat uterus (5). For this purpose, virgin albino female rats (150-180 g) were injected twice with 0.2 mg/kg of stilbestrol (F.W. Horner, Ltd., Montreal, Canada) 48 and 24 hr prior to the assay. The animals were sacrificed and the horns of the uterus removed. One of the horns was opened lengthwise and suspended in a 5.0-mL tissue bath containing oxygenated de Jalon solution, pH 7.4, at 29°C, with 1.0 µg/mL atropine sulfate (Sigma Chemical Co., St. Louis, MO). The uterus was standardized with synthetic bradykinin (Sandoz, Montreal, Canada) prepared in concentrations 0.2 to 0.6 ng/mL. Standards were applied to the uterus every 5 to 6 min and the height of contractions produced by the standards were recorded on a kymograph (Palmer, London, England) by a lever attached to the uterus. A standard curve was prepared by plotting the height of the contractions against the logarithm of the concentration of the bradykinin (BK) standards. The experimental samples were diluted to give concentrations lying within the dose-response range and the height of the contractions was compared with the known standards.

The total concentration of kinin (ng/mL) present was calculated by multiplying the dilution factor by the equivalent response obtained (5). The range of variation from the duplicate samples was < 5%.

Results

Effect on Partial Thromboplastin Time

Blood remains fluid within the body but quickly clots when placed in contact with a negatively charged surface, such as glass, quartz or kaolin (6). The series of reactions, leading to the clotting of blood, was called the cascade of coagulation by MacFarlane (7) and can be described as in Figure 1. Contact of the blood with certain surfaces activates factor XII (Hageman factor) which activates factor XI, which in turn activates factor IX. Activated factor IX, in conjunction with phospholipid, calcium and factor VIII, activates factor X. Activated factor X, in association with phospholipid, calcium, and factor V, causes the rapid conversion of prothrombin to thrombin. Platelets are the main source of the phospholipid to which these clotting factors can bind.

We have shown (8) that both the negatively charged amphiboles (IARC crocidolite and IARC amosite) and the positively charged chrysotiles

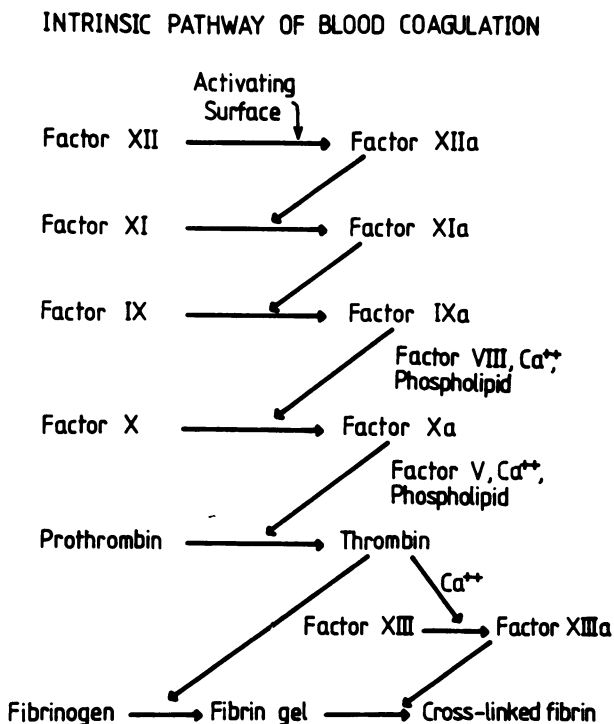


FIGURE 1. Intrinsic pathway of blood coagulation. The designation "a" following the Roman numeral for a clotting factor indicates the active form.

(IARC chrysotile A and IARC chrysotile B) shorten the PTT of human plasma, indicating acceleration of coagulation and, at the concentration used (10 mg/mL), were comparable to the control samples, kaolin and quartz (Table 1).

Activation of Factor XII

The activation of factor XII by contact activation is in fact more complicated than shown in Figure 1 and, in addition, the kinin-forming and the fibrinolytic pathways are triggered. A milestone in the development of our present concepts was the discovery of Fletcher trait plasma, deficient in plasma kallikrein, and Fitzgerald trait plasma, deficient in high molecular weights (HMW) kininogen. A proposed mechanism for factor XII activation is illustrated in Figure 2 (1, 11). The activation of bound factor XII to an activating surface is greatly enhanced by HMW-kininogen which is also capable of binding to the surface. Since HMW-kininogen circulates in plasma with prekallikrein and factor XI, this interaction helps to localize factor XII substrates. The generated factor XIIa (Fig. 1) and plasmin participate in intrinsic coagulation and in fibrinolysis, respectively. Kallikrein, on the other hand, cleaves kininogens to generate kinins and also clips factor XII which further activates prekallikrein to kallikrein.

The results in Figure 3 indicate that only the negatively charged fibers, amosite and crocidolite, are capable of activating factor XII, in the presence of HMW-kininogen, to generate kallikrein. This behavior is similar to that of the negatively charged standards, kaolin and quartz.

From Figure 4 it can be seen that crocidolite generates kinin from normal plasma but not from factor XII-deficient plasma (Hird) and HMW-kininogen-deficient plasma (Williams). The maximum kinin genera-

tion was obtained after 5 min incubation using normal human plasma. These results are typically obtained with the commonly used standard kaolin (4).

Discussion

Contact between asbestos fibers and plasma components could possibly result, for example, after fiber-mediated damage of bronchial or alveolar epithelial surfaces (12); alternatively, fibers might be released from damaged alveolar macrophages (13) after transport into peribronchial areas and lymphatic capillaries. It is proposed that the above findings might be relevant to the inflammatory processes associated with asbestosis for the following reasons. The deposition of fibrin is likely to contribute, in general, to the induration characteristic of inflammatory responses, such as delayed type hypersensitivity reactions (14); in addition, cells such as macrophages, which have receptors for fibrin (15), might be trapped at a site by a fibrin deposit. Kinins have been shown to have certain activities relevant to inflammation, for example, dilation of blood vessels, increase in vascular permeability and pain induction (1). In this context, Suzuki and Churg (12) have described the exudation of edema fluid in alveolar spaces soon after intratracheal injection of chrysotile fibers into hamsters (indicating an increase in vascular permeability), vascular modifications including venous dilation have been elicited in the hamster cheek pouch by asbestos fibers (16) and the hyperemia induced in rabbit skin by asbestos fibers has been quantitated (17). In addition

SURFACE-MEDIATED KININ GENERATION

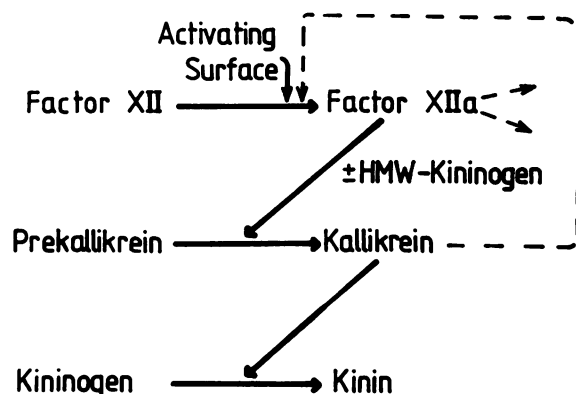


FIGURE 2. Proposed mechanism for the effect of activation of factor XII on the activation of prekallikrein. The product of prekallikrein activation, namely kallikrein, can, in turn, augment the activation of factor XII while HMW kininogen promotes the activation of factor XII by surfaces [--->, denotes the effect of factor XIIa on blood coagulation (Fig. 1) and on the activation of plasminogen to plasmin leading to fibrinolysis].

Table 1. Effect of asbestos fibers on the partial thromboplastin (PTT) time of normal human plasma.^a

Test substance	Clotting time, sec
IARC crocidolite ^b	60
IARC amosite	61
IARC chrysotile A	69
IARC chrysotile B	104
Calidria chrysotile ^c	357
Quartz	67
Kaolin	57
Buffer	234

^a Data from Hamilton et al. (8).

^b The physicochemical characteristics of the IARC-standardized asbestos fibers have been reported (9).

^c Calidria chrysotile pellets were ultrasonicated for 2 min to disaggregate the chrysotile to the smallest fibers. The physicochemical properties of this material have been described (10). Approximately 95% of the fibers were calculated to be less than 5 μ m in length (10).

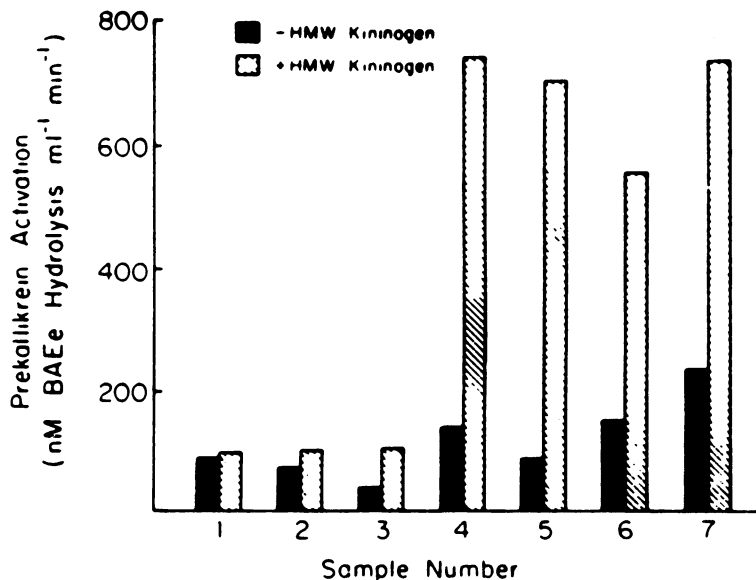


FIGURE 3. Activation of factor XII by negatively charged asbestos fibers. The range of variation from duplicate samples was <5%. Sample numbers were as follows: (1) IARC chrysotile B; (2) IARC chrysotile A; (3) Calidria chrysotile; (4) IARC amosite; (5) IARC crocidolite; (6) quartz; (7) kaolin.

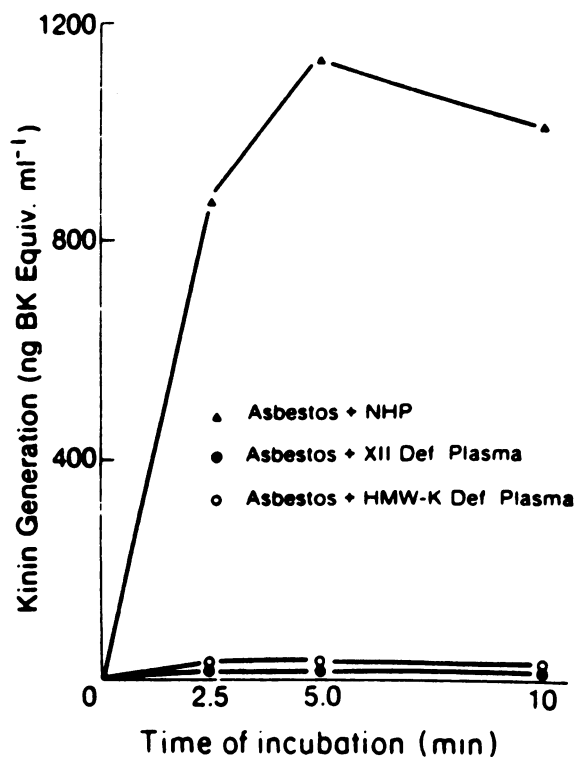


FIGURE 4. Generation of kinin by crocidolite (8).

to fibrin deposition and kinin liberation, the activation of factor XII can lead to the activation of the complement system, plasmin formation and the generation of bioactive fragments from fibrinogen and fi-

brin (1). Vascular changes have been suggested as possibly being relevant to the cocarcinogenic properties (tumor promotion) of asbestos fibers (17).

The ability of the positively charged chrysotiles to shorten the PTT was surprising, given that negatively charged surfaces are generally required for activation of the intrinsic coagulation pathway via factor XII activation (18). Perhaps another coagulation sequence is activated. Also, it would be of interest to know whether chrysotiles (in addition to amphiboles) can generate kinins, even though the samples studied here did not activate factor XII. The sample of Calidria chrysotile (with 95% fibers <5 μ m in length) had minimal PTT activity; further studies could determine what fiber dimensions are critical in the interaction with plasma. It is important that the properties of short asbestos fibers be studied since they are used extensively but are not currently included in safety standards due to the difficulty of detection.

A cigarette smoke glycoprotein has been shown to activate factor XII to generate clotting and kinin activities (19). It was proposed in this article that the end result would be vascular effects similar to those described above and therefore might be important in the pathogenesis of pulmonary disease associated with cigarette smoking. It is possible that the findings reported above and those of Becker and Dubin are relevant to the well-established synergism between cigarette smoking and asbestos inhalation in the incidence of asbestosis (20).

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